## Docket No.:10031294

## **CLAIMS**

## What is claimed is:

- 1. A method of preparing an RNA sample substantially free of genomic DNA, comprising the following steps:
  - (a) forming a tissue/cell lysate from a biological sample;
  - (b) removing essentially all of the gDNA;
- (c) forming a precipitate by adding an organic solvent to the preparation of (b);
- (d) contacting an RNA isolation membrane column with said precipitate of (c), wherein said column comprises a membrane; and
- (e) collecting said precipitate from said membrane, wherein said precipitate is substantially free of said genomic DNA.
- 2. The method of claim 1, wherein said membrane is a polymeric membrane.
- 3. The method of claim 2, wherein said polymeric membrane is selected from the group consisting of BTS, PVDF, nylon, nitrocellulose, polysulfone, MMM, PVP, and composites thereof.
- 4. The method of claim 1, wherein said membrane has a particle retention ranging from about 0.1 to about 10  $\mu$ m.
- 5. The method of claim 1, wherein said step (b), removing essentially all of the gDNA, is accomplished by using a pre-filtration technique.
- 6. The method of claim 1, wherein said lysate is formed employing a lysis buffer comprising a chaotropic agent.

7. The method of claim 6, wherein said chaotropic agent is selected from a group consisting of guanidine isothiocyanate, ammonium isothiocyanate, guanidine hydrochloride and combinations thereof.

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- 8. The method of claim 7, wherein said chaotropic agent is at a concentration ranging from about 0.5 M to about 5.0 M.
- 9. The method of claim 1, wherein said biological sample is selected from the group consisting of animal and plant tissues and/or cells.
- 10. The method of claim 9, wherein said animal tissues and/or cells are selected from a group consisting of blood, urine, hair, skin, muscle, bone, bodily fluids, organ extracts and alike.
- 11. The method of claim 1, wherein step (e) is followed by the use of DNase treatment.
- 12. The method of claim 1, wherein said precipitate comprises RNA essentially free of DNA.
- 13. The method of claim 1, wherein said lysate is formed using a lysis buffer comprising  $\beta$ -mercaptoethanol.
- 14. The method of claim 1, wherein said organic solvent is an alcohol selected from the group consisting of methanol, ethanol, isopropanol and combinations thereof.
- 15. The method of claim 1, wherein said precipitate is washed following step (d) with a wash solution comprising an organic solvent.
- 16. The method of claim 15, wherein said wash solution is selected from the group consisting of Wash Buffer #1 and Wash Buffer #2.

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- 17. The method of claim 16, wherein said Wash Buffer #1, comprises:
  - (a) from about 0.2 to about 2 M guanidine;
  - (b) from about 5 to about 25% ethanol; and
  - (c) a buffering agent to maintain a pH from about 6 to about 9.
- 18. The method of claim 16, wherein said Wash Buffer #2, comprises:
  - (a) from about 40 to about 90% ethanol; and
  - (b) a buffering agent to maintain a pH from about 6 to about 9.
- 19. A method of preparing an RNA sample substantially free of genomic DNA, comprising the following steps:
  - (a) forming a tissue/cell lysate from a biological sample;
- (b) contacting a pre-filtration column with said lysate, wherein said pre-filtration column comprises a fiber material, wherein said fiber material has at least one layer of glass or borosilicate fiber;
  - (c) forming a precipitate by adding an organic solvent to step (b);
- (d) contacting an RNA isolation membrane column with said preparation from step (c), wherein said RNA isolation membrane column comprises a membrane; and
- (e) collecting said precipitate from said RNA isolation membrane column, wherein said precipitate is substantially free of said genomic DNA.
- 20. The method of claim 19, wherein said fiber material has a particle retention ranging from about 0.1  $\mu m$  to about 10  $\mu m$ .
- 21. The method of claim 19, wherein said fiber material has a thickness ranging from about 50  $\mu m$  to about 2000  $\mu m$ .
- 22. The method of claim 19, wherein said fiber material has a specific weight ranging from about 75 g/m<sup>2</sup> to about 300 g/m<sup>2</sup>.
- 23. The method of claim 19, wherein said RNA isolation membrane has a particle retention ranging from about 0.1 to about 10 µm.

24. The method of claim 19, wherein said RNA isolation membrane is selected from the group consisting of BTS, PVDF, nylon, nitrocellulose, polysulfone, MMM, PVP, and composites thereof.

- 25. A kit for isolating RNA in a form essentially free from gDNA, comprising the following:
- (a) at least one pre-filtration column, wherein said pre-filtration column comprises a fiber material, wherein said fiber material has at least one layer of glass or borosilicate fiber;
- (b) at least one RNA isolation membrane column, wherein said membrane column comprises a membrane;
  - (c) reagents for both (a) and (b); and

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- (d) instructions for implementing (a) through (c).
- 26. The kit of claim 25, wherein said RNA isolation membrane is selected from the group consisting of BTS, PVDF, nylon, nitrocellulose, polysulfone, MMM, PVP, and composites thereof.
- 27. The kit of claim 25, wherein said reagents include at least one organic solvent and a lysis buffer.